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The Role of Platelet Activation and Inflammation in Early Brain Injury following Subarachnoid Hemorrhage

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Abstract

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SUPPLEMENTARY MATERIAL

There is one supplementary figure.

Introduction: Early brain injury (EBI) following aneurysmal subarachnoid hemorrhage (SAH) is an important predictor of poor functional outcome, yet the underlying mechanism is not well understood. Animal studies suggest that platelet activation and inflammation, with subsequent microthrombosis and ischemia may be a mechanism of EBI.

Methods: A prospective, hypothesis-driven study of spontaneous, SAH patients and controls was conducted. Platelet activation (thromboelastography maximum amplitude [MA]) and inflammation (C-reactive peptide [CRP]) were measured serially over time during the first 72h following SAH onset. Platelet activation and inflammatory markers were compared between controls and SAH patients with mild (Hunt-Hess [HH] 1-3) versus severe (HH4-5) EBI. The association of these biomarkers with 3-month functional outcomes was evaluated.

Results: We enrolled 127 patients (106 SAH; 21 controls). Platelet activation and CRP increased incrementally with worse EBI/HH grade and both increased over 72h (all $P < 0.01$). Both were higher in severe versus mild EBI (MA 68.9 versus 64.8mm, $P = 0.001$; CRP 12.5 versus 1.5mg/L, $P = 0.003$) and compared to controls (both $P < 0.003$). Patients with delayed cerebral ischemia (DCI) had more platelet activation (66.6 versus 64.9 in those without DCI, $P = 0.02$) within 72h of ictus. At 3-months, death or severe disability was more likely with higher levels of platelet activation (mRS4-6 OR 1.18, 95% CI 1.05-1.32, $P = 0.007$) and CRP (mRS4-6 OR 1.02, 95% CI 1.00 – 1.03, $P = 0.041$).

Conclusions: Platelet activation and inflammation occur acutely after SAH and are associated with worse EBI, DCI and poor 3-month functional outcomes. These markers may provide insight into the mechanism of EBI following SAH.

Keywords

early brain injury; inflammation; microthrombosis; platelet; SAH; subarachnoid hemorrhage

INTRODUCTION:

Admission neurological status after subarachnoid hemorrhage (SAH) reflects the severity of early brain injury and is a larger predictor of death or severe disability than any other factor or complication that occurs as a consequence of aneurysm rupture.^{1, 2} However, the mechanisms for early brain injury are not well understood, and no effective therapeutic intervention currently exists.

In both animal and human models, SAH leads to transiently elevated intracranial pressure (ICP) with concomitant inadequate cerebral blood flow and perfusion, and in severe cases intracranial circulatory arrest^{3, 4}. This transient global hypoperfusion (often evidenced by loss of consciousness at ictus) initiates a cascade of events that include diffuse upregulation of endothelial adhesion molecules, platelet activation, and inflammation, that culminate in microthrombosis, ischemia, vasogenic edema and early brain injury⁵⁻⁷. Platelets amplify the inflammatory cascade⁸. Once activated, platelets display P-selectin and release chemokines/cytokines that promote leukocyte adherence and transmigration at sites of platelet deposition⁸. Neutrophils also release factors that promote platelet activation⁹. Activated platelets and inflammatory cells can contribute to further endothelial disruption, perpetuating the cycle of microthrombosis and inflammation, even distant from the aneurysm rupture site^{5, 7, 10, 11}.

Indeed, we have shown in pilot data that measures of platelet activation and markers of inflammation (C-reactive peptide) were higher in SAH patients with more severe early neurological injury.¹²

In this larger prospective, controlled cohort study, we hypothesize that platelet activation and inflammation will be increased in SAH patients compared to controls and associated with clinical evidence of early brain injury, and worse long-term functional outcomes.

METHODS:

Study Population

A prospective, hypothesis driven study was conducted of patients with spontaneous SAH between 4/2010-6/2015 at two tertiary care neuro-intensive care units at Mount Sinai Hospital (MSH) in New York and Cleveland Clinic (CC). Consecutive patients were enrolled at each institution as the PI (Frontera) moved from MSH to CC, with an intervening six month start-up time at CC. Study inclusion criteria were defined as: spontaneous SAH diagnosed by head computed tomography or lumbar puncture, enrolment within 72 hours of hemorrhage onset (prior to the onset of delayed cerebral ischemia or vasospasm), initial blood sampling prior to catheter angiography or aneurysm repair, informed consent and age 18 years. Exclusion criteria were defined as: secondary SAH (from trauma, dissection, amyloid angiopathy, Moya Moya, vasculitis, arteriovenous malformation), clinical or radiographic evidence of ultra-early vasospasm or delayed cerebral ischemia (DCI), use of irreversible platelet inhibitors within 7 days, use of reversible platelet inhibitors within 4 half-lives of the first blood draw, intrinsic platelet dysfunction, thrombocytopenia (platelet count <100 per μ L), platelet transfusion, use of immunosuppressant drugs including steroids, or known immunocompromised state.

A control group consisted of antiplatelet naïve patients with known unruptured intracranial aneurysms and planned elective, prophylactic aneurysm repair via either clipping or coiling. This group was selected so that we could control for platelet activation and inflammation that may occur with aneurysm formation or following an aneurysm repair procedure. The control group was subjected to the same exclusion criteria as the SAH group. The study was approved by the Mount Sinai and Cleveland Clinic Institutional Review Boards (IRB). All patients or their surrogates were consented for participation in this study.

Laboratory Studies

Venous blood was sampled serially at three time points: 0-24, 24-48 and 48-72 hours of aneurysm rupture, prior to the clinical or radiographic onset of delayed cerebral ischemia (vasospasm), which typically occurs within 3-14 days following aneurysm rupture¹. In a subgroup of SAH patients, simultaneous jugular and peripheral venous sampling was performed to compare measurements representative of cerebral venous drainage (jugular bulb catheter sampling) to peripheral venous samples (which could be contaminated by systemic inflammation related to critical illness). Control patients underwent venous blood testing before, and 24 hours after elective aneurysm repair. The initial blood sample in SAH and control patients was collected prior to digital subtraction angiography and/or aneurysm

repair to evaluate for platelet activation or inflammation that may be induced as part of the aneurysm repair process.

Platelet activation was measured using Thromboelastography (TEG; Haemoscope Corporation, IL, USA). Thromboelastography (TEG) is an FDA approved point-of-care device that measures the speed and strength of clot formation. The TEG parameter MA (maximum amplitude) measures platelet and fibrin contribution to clot formation¹³. Elevated TEG MA indicates clot strength and is a marker of platelet activation¹³⁻¹⁶ (normal range 51.0-69.0 mm).

Inflammation was assessed by measuring C-reactive protein (CRP) and the %neutrophils in venous blood collected at 0-24, 24-48 and 48-72 hours after aneurysm rupture. Blood samples for all biomarkers of platelet activation and inflammation were collected at the same time.

Clinical and Outcome Measures

The time of aneurysm rupture was recorded as the patient reported time of first headache or the time last known at baseline, if the headache onset time was unknown. Early brain injury (EBI) was assessed at admission using the Hunt-Hess grade^{17, 18}. Admission neurological severity was dichotomized as severe EBI (Hunt-Hess 4-5) versus mild EBI (Hunt-Hess 1-3). To corroborate our findings, other admission measures of neurological injury were also assessed including the Glasgow Coma Scale (GCS)¹⁹, and the National Institute of Health Stroke Scale (NIHSS)²⁰. All neurological batteries were performed *after* resuscitation (including external ventricular drain placement) to eliminate confounding factors that may adversely affect the neurological exam. Delayed cerebral ischemia was defined as clinical neurological deterioration accompanied by radiographic/angiographic evidence of reversible vessel narrowing or infarction detectable by CT or MRI due to vasospasm.¹

Functional outcomes were assessed at 3-months via phone interview using the modified Rankin Scale dichotomized as mRS 0-3 (good functional outcome) versus 4-6 (poor outcome).^{21, 22}

Statistical Analysis

The sample size for this study was calculated to detect a difference in platelet activation (thromboelastography MA values) between patients with mild (Hunt-Hess 1-3) and severe (Hunt-Hess 4-5) EBI. Preliminary data showed that mild and severe Hunt-Hess grade patients were admitted in a ratio of 4:1. Based on pilot data showing a mean MA of 65.4 mm and a standard deviation of 5.0 mm, a sample size of 110 patients (88 mild and 22 severe) would be required to detect a 4 mm difference in MA with 80% power and 0.05 significance level. *A priori*, a minimum of 20 patients were selected for the control group. This number of controls was selected due to the confines of our budget. Additionally, our study was not designed to test SAH versus controls, but rather to distinguish mild from severe EBI early after SAH. Hence, our power calculation did not account for control subjects.

Demographic and repair procedure variables were compared between the SAH and control group using Chi-Squared analysis for categorical variables and Mann-Whitney U tests for

continuous variables. The median platelet activation, and inflammatory biomarker values (over the first 72 hours from ictus in SAH patients and pre-aneurysm repair in controls) and were compared in the following groups: 1) across three groups: controls, mild EBI SAH (Hunt-Hess 1-3) and severe EBI (Hunt-Hess 4-5) SAH patients, 2) all SAH patients versus controls, 3) severe EBI SAH patients versus controls and 4) severe versus mild EBI SAH patients using the Mann-Whitney U and Kruskal-Wallis tests. The median platelet activation, CRP and %neutrophils were compared over each individual level of the Hunt-Hess scale using ordinal logistic regression. Additionally, a Jonckheere-terpstra test for trend was used to assess the “dose-response” relationship between platelet activation, CRP and %neutrophil values and each level of the Hunt-Hess grade. Backwards, stepwise logistic regression analysis was used to evaluate the association of platelet activation, CRP and %neutrophils with EBI after adjusting for covariates. Repeated measures analyses (two way repeated measures analysis of variance [ANOVA] using one factor repetition) were used to assess the changes in platelet activation, CRP and percent neutrophils over time. The correlations among jugular and peripheral venous measures of platelet activation, CRP, and percent neutrophils were assessed using Spearman’s correlation coefficient. Logistic regression was used to assess the association between platelet activation, CRP, percent neutrophils and 3-month dichotomized mRS. All analyses were performed using commercially available SPSS (v. 21, Chicago IL, USA) and SAS (v 9.3, Cary, NC, USA) statistical software. Significance was set at $P < 0.05$.

RESULTS:

A total of 306 patients with SAH were screened for enrolment: 57 were excluded for prior antiplatelet or immunosuppressant use, 8 were excluded after receiving a platelet transfusion at admission, 71 had non-aneurysmal causes of SAH (most commonly trauma), 41 were admitted outside of the time window (over 72h from ictus), 12 refused consent, and 11 were not enrolled for other reasons. A total of 106 SAH patients (26 from MSH and 80 from CC) and 21 controls (all from CC) met inclusion criteria and were enrolled. Among SAH patients, 24 (23%) had severe EBI (Hunt-Hess 4-5) and 82 (77%) had mild EBI (Hunt-Hess 1-3). All 127 subjects underwent platelet activity testing (thromboelastography) and an additional 77 (59 SAH and 18 control) had CRP and % neutrophil testing, which was initiated after the pilot phase of the study. The SAH and control groups were similar in age, although there was a higher proportion of women, and aneurysm clipping was more common in the control group (Table 1).

SAH patients with severe EBI (Hunt-Hess grade 4-5) had significantly higher levels of platelet activation and CRP (averaged over 72h from ictus) compared those with mild EBI (Hunt-Hess 1-3) patients and controls (Table 2). Similarly, platelet activation and markers of inflammation, including C-reactive peptide (CRP) and the %neutrophils increased across each individual level of the Hunt-Hess scale (Figure 1). A “dose-response” effect was demonstrated such that each level increase of the Hunt Hess scale was associated with incrementally higher platelet activation (Jonckheere-Terpstra Z statistic 4.0, $P < 0.001$), and CRP (Jonckheere-Terpstra Z statistic 3.4, $P = 0.001$). To corroborate these findings, we also examined associations with other clinical neurological scales and found worse median admission Glasgow Coma Scores and NIHSS scores in patients with higher (greater than

median) levels of platelet activation (GCS 13 with higher platelet activation versus 15 in lower platelet activation, $P=0.004$; and NIHSS 7 versus 0, $P=0.001$). Similarly, patients with elevated CRP had worse median admission GCS (10 versus 15, $P=0.002$) and NIHSS (9 versus 0, $P=0.001$). Over the first 72h from ictus, platelet activation was significantly correlated with markers of inflammation including CRP (Spearman's correlation coefficient $R=0.599$, $P<0.001$), and %neutrophils (Spearman's correlation coefficient $R=0.311$, $P=0.036$). Percent lymphocytes and %monocytes were not significantly different among SAH patients with mild or severe EBI or compared to controls. Additionally, there was no evidence of disseminated intravascular coagulopathy (DIC) or platelet dysfunction among any of the SAH patients (based on TEG LY30 and MA values).

We evaluated possible confounding conditions that might cause platelet activation or inflammation including the occurrence of meningitis/ventriculitis, pneumonia, urinary tract infection, blood stream infection, seizure, myocardial infarction, neurogenic stunned myocardium, pulmonary edema and intubation. Only intubation was associated with elevated platelet activation (67 mm versus 65 mm in those not intubated, $P=0.013$). Pneumonia was associated with elevated CRP (6.6 mg/dL versus 1.4 mg/dL in those without pneumonia, $P=0.009$) and %neutrophils (85% versus 80%, $P=0.003$). Pulmonary edema was also associated with elevated CRP (4.8 mg/dL versus 1.1 mg/dL in those without pulmonary edema, $P=0.027$) and %neutrophils (84% versus 80%, $P=0.046$); as was intubation (CRP 6.4 mg/dL versus 0.5 mg/dL in those not intubated and %neutrophils 84% versus 79%, $P=0.016$). None of the other conditions were significantly associated with platelet activation, CRP or %neutrophils. Using backward stepwise logistic regression analysis adjusting for intubation, platelet activation was still significantly associated with EBI (adjusted OR 1.13, 95% CI 1.0-1.3, $P=0.049$).

Patients with increased platelet activation within 72 hours of ictus had a higher likelihood of developing delayed cerebral ischemia (DCI) later during their hospitalization (median MA 66.6 mm in those who developed DCI versus 64.9 mm in those who did not, (OR for developing DCI with increasing platelet activation: 1.1, 95% CI 1.0-1.2, $P=0.02$). Early elevations in CRP and %neutrophils over the first 72 hours from ictus were not associated with DCI. There was no association of age, gender or the type of aneurysm repair procedure (clipping or coiling) with platelet activation, CRP or %neutrophils.

Over the first 72 hours following aneurysm rupture, both platelet activation and CRP increased over time ($P<0.0001$ and $P=0.001$, respectively; Figure 2), but the %neutrophils had a non-significant trend toward decreasing over time ($P=0.07$).

The effects of the aneurysm repair procedure on platelet activation and inflammation were evaluated (Figure 3). There was no change in platelet activation in the control group after aneurysm repair (median pre-repair MA 64.9 mm and post-repair 64.9, $P=0.87$) and there was a small increase in the SAH group (median pre-repair MA 64.9 mm to 65.3 mm post repair, $P=0.047$), though this may not be clinically significant. Both CRP and %neutrophils increased modestly from pre- to post-aneurysm repair in controls (median pre-repair CRP 0.3 mg/L to 1.5, $P<0.001$; median pre-repair %neutrophils 63% to 83.5% post repair, $P=0.002$) and CRP increased in SAH patients (median pre-repair CRP 0.5 mg/L to 2.8 mg/L

post repair, $P=0.003$) though %neutrophils did not (median pre-repair %neutrophils 84% to 81.6% post repair, $P=0.892$). However, post-aneurysm repair platelet activation, and CRP were still significantly higher in poor grade SAH patients compared to controls (median post-repair MA 69.2 mm in Hunt-Hess 4-5 SAH versus 64.9 mm in controls, $P=0.007$; median post-repair CRP 17.6 mg/L in Hunt-Hess 4-5 SAH versus 1.5 mg/L in controls, $P<0.0001$)

In a secondary analysis, jugular and peripheral venous levels of platelet activation and inflammation were compared. This subanalysis was done to determine if there were substantial differences between jugular venous blood (which represents almost exclusively cerebral venous drainage) and peripheral venous sampling (which could be contaminated by systemic inflammation related to critical illness). A total of 40 patients underwent simultaneous jugular and peripheral venous sampling every 24 hours for three days following aneurysm rupture. During the first 72 hours from ictus, jugular and venous MA, CRP and %neutrophils were very similar, though jugular levels were slightly higher (supplementary Figure 1). There was a significant correlation between jugular and venous platelet activation, CRP and %neutrophils (Spearman's correlation coefficient $R=0.92$, 0.99 , and 0.90 respectively, all $P<0.0001$). Similar increases in jugular and peripheral venous MA and CRP levels occurred over time (supplementary Figure 1).

Three month follow-up data was available in 92/106 (87%) of SAH patients. Early platelet activation was significantly associated with increased 3-month mortality (Odds Ratio [OR] for mortality with increasing platelet activation: 1.18, 95% confidence interval [CI] 1.04-1.34, $P=0.011$) and worse 3- month functional outcomes (OR for mRS 4-6 with increasing platelet activation: 1.18, 95% CI 1.05-1.32, $P=0.007$; Table 3.). Three month death or severe disability was also associated with higher markers of inflammation (OR for mRS 4-6 with increasing CRP: 1.02, 95% CI 1.00 –1.03, $P=0.041$).

DISCUSSION:

This study is one of the first, and largest, prospective clinical studies to test the hypothesis that acute elevations in platelet activation and inflammation are associated with early brain injury following spontaneous SAH. We found that SAH patients with clinical evidence of severe brain injury (measured by the Hunt-Hess grade and corroborated by the NIHSS and GCS scales) had significant elevations in platelet activation and inflammation compared to less severely affected SAH patients (Hunt-Hess 1-3) and controls within 72 hours of ictus. Furthermore, there was a “dose-response” effect with incrementally increasing platelet activation and inflammation as Hunt-Hess grade and EBI worsened. Even after controlling for other factors, platelet activation was independently associated with EBI. These biomarkers were not only associated with EBI following SAH, but also with worse 3-month functional outcomes.

Our clinical data corroborates a mechanism for early brain injury in SAH elucidated in animal studies. In a rat endovascular perforation model of SAH²³, platelet aggregates have been observed to occur in microvessels within 10 minutes of SAH and continue to increase over the first 48 hours from vessel rupture.^{5, 24} Platelet aggregates may cause

microthrombosis, ischemia and resultant tissue death. Indeed, endothelial and parenchymal apoptosis and neuronal necrosis occur in animals within 10 minutes of SAH and continue to increase over 24 hours from ictus, concomitant with platelet aggregation within microvessels.²⁵ In humans, we and others have demonstrated that MRI-detected infarctions occur acutely after SAH (and prior to the onset of DCI)²⁶. These infarctions occur more commonly in patients with more severe EBI (Hunt-Hess 4-5) and are associated with an increased risk of DCI and worse 3-month functional outcomes.²⁶⁻²⁸ Such infarctions may be the result of small vessel microthromboses, particularly since 75% of were observed to be punctate, occurring at the small vessel level.²⁶ An autopsy study of 7 patients who died within 3 days after SAH found a high microclot burden associated with pathological evidence of ischemia, indicating that microthromboses occur acutely and may be a mechanism of early brain injury after SAH.²⁹

Platelet activation occurs concomitantly with inflammation. This was demonstrated in our study by simultaneous elevations in CRP, %neutrophils and platelet activation in SAH patients compared to controls, and significant correlation coefficients. In animal models, intraluminal platelets initiate or stimulate endothelium and basal lamina disruption, as evidenced by acute loss of collagen IV³⁰ and increased vessel permeability.¹⁰ Resultant blood brain barrier breakdown and inflammation is evidenced by escape of platelet aggregates and neutrophils into the brain parenchyma^{6, 11}. Several other clinical studies have identified an increase in immunologic and inflammatory markers in patients with both unruptured intracranial aneurysms and subarachnoid hemorrhage.^{31, 32} We found that platelet activation and CRP increased over the first 72 hours from ictus. Though %neutrophils were initially elevated in SAH patients compared to controls, this biomarker tended to decline over time. This may be due to diapedesis of neutrophils into the brain parenchyma as suggested by animal models, though further study is required to support this hypothesis. It is possible that platelet activation and inflammation may continue to rise during the DCI/vasospasm risk period. Future studies are needed to test this hypothesis.

While inflammation and platelet activation have been reported in association with DCI³³, our study is one of the first to examine an association with EBI. For decades, clinical research in SAH has focused on preventing and treating secondary injury after SAH (i.e. DCI/vasospasm), while EBI has been understudied³⁴. Multiple groups have found an association of elevated platelet activating factor (PAF), platelet aggregability, platelet microparticles (CD41+), elevated thromboxane levels, and microthrombosis with the development of DCI^{33, 35}. Autopsy reports have also found diffuse microthromboses in patients who died from cerebral vasospasm following SAH.^{29, 36, 37} In fact, the cascade of injury that begins at the time of aneurysm rupture and causes EBI, may be in continuum with the pathophysiological events that lead to DCI. Our study identified a significant association of elevated platelet activation and CRP within the first 72 hours of ictus with the development of DCI.

To validate the importance of early platelet activation and inflammation, we were able to demonstrate a significant association with poor 3-month functional outcomes, though the association of CRP with 3-month outcomes is small and may not be clinically significant. In smaller cohort studies, others have found that early elevations in biomarkers of inflammation

(TNF α , serum neutrophils) and platelet activation (CD41+ platelet microparticles) have been associated with poor outcomes following SAH.³⁸⁻⁴⁰

Additional strengths of this study include the utilization of control patients and the correlation of peripheral and jugular blood biomarkers. First, the control group allowed us to calibrate the effects of aneurysm repair on platelet activation and inflammation and also to adjust for any inflammatory process that may occur as part of aneurysm development.³¹ We found modest elevations in platelet activation and CRP after aneurysm repair, but levels were still significantly lower in controls compared to SAH patients with evidence of severe early brain injury. Second, we were able to verify that peripheral blood sampling was highly correlated with jugular blood measurements, which are directly representative of the cerebral milieu. Because the jugular levels of platelet activation and inflammation were slightly higher than the peripheral levels and because the primary cause of critical illness is brain injury due to SAH, we believe that the peripheral platelet activation and inflammation levels directly reflect cerebral processes.

Implications of this study are that early platelet inhibition may have a protective effect in SAH patients. Platelet inhibition has been found to attenuate platelet activation and PLA formation in animal models of SAH.⁴¹ However, human studies of SAH have shown mixed results with antiplatelet use. A randomized study of aspirin to prevent delayed cerebral ischemia did not show benefit, though aspirin was started late (median of 4 days from ictus) and the study was underpowered to examine functional outcomes.⁴² In a study of 226 SAH patients undergoing endovascular coiling, antiplatelet drugs (either aspirin or abciximab in varying doses) did not show any difference in outcome at 2 months or 1 year compared to coiled patients who did not receive an antiplatelet.⁴³ A limitation of the study was that only 56 patients received antiplatelets during coiling, while the rest were prescribed antiplatelets at varying times after coiling. Therefore, the time to dosing of antiplatelets may not have been within a meaningful time window to prevent acute brain injury. Another study noted that baseline aspirin use in patients with known aneurysms was associated with lower rates of aneurysm rupture.⁴⁴ Though there was no difference in admission clinical status or outcome of those on aspirin compared to aspirin naïve patients, all patients on aspirin received a platelet transfusion at admission, which may have attenuated any positive aspirin effects. A meta-analysis of 5 trials and 699 patients found a trend towards improved outcomes in patients who received antiplatelet agents, though the time to initiation of an antiplatelet ranged from within 48 hours (only 13 patients) to 9 days from ictus.⁴⁵ Given the limitations in the current literature, further exploration of the early effects of aspirin on the proposed mechanism of acute brain injury after SAH may be useful.

Certain limitations of this study bear mentioning. First, we can only demonstrate an association of platelet activation and inflammation with EBI and worse long-term outcomes; we cannot prove causality. However, we did observe an incremental “dose-response” increase in platelet activation and inflammation as the Hunt-Hess grade and EBI severity increased, which may suggest more than just an association. Second, activated platelets and inflammatory cells may be primarily adherent to the endothelium of small vessels and not shed in the blood. We assume that elevated circulating activated platelets and markers of inflammation correlate with elevated levels of platelets and leukocytes adherent to brain

microvessel endothelium. Since our data shows a strong correlation between peripheral and jugular venous blood measurements (with jugular levels being slightly higher), it is reasonable to assume that the biomarkers we examined represent the cerebral milieu. Third, this study was not powered to assess the effect of early platelet activation and inflammation on long-term functional outcomes after adjusting for other covariates. Hence, we did not perform a multivariable analysis examining the predictive effect of platelet activation and inflammation on 3-month mRS. Finally, because only 60% of our patients underwent CRP and %neutrophil testing, this limited our power to detect the impact of inflammation on 3-month outcomes. Furthermore, CRP and %neutrophils are relatively non-specific markers of inflammation. Future studies evaluating cytokine/chemokine inflammatory markers may provide more insight into the mechanisms of EBI.

CONCLUSIONS:

Increased markers of platelet activation and inflammation occur acutely after SAH and are associated with clinical evidence of early brain injury, delayed cerebral ischemia and worse 3-month functional outcomes on univariate analysis. Further research exploring the mechanistic role that these biomarkers may play in early brain injury after SAH is warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Platelet activation (thromboelastography [TEG] MA values) and markers of inflammation (C-reactive protein and percent neutrophils) in controls and across each level of the Hunt-Hess scale. Levels represent averages over the first 72 h from SAH ictus. *P* values were derived from ordinal logistic regression analysis. *Whisker bars* represent the standard error of the mean. *MA* maximum amplitude; *CRP* C-reactive protein

Fig. 2. Platelet activation, C-reactive protein (CRP) and percent neutrophils over the first 72 h following aneurysm rupture. *Whisker bars* represent the standard error of the mean. *MA* maximum amplitude, *TEG* thromboelastography

Fig. 3.

Effect of the aneurysm repair procedure on platelet activation, C-reactive protein (CRP) and percent neutrophils. *Whisker bars* represent the standard error of the mean. *MA* maximum amplitude, *SAH* subarachnoid hemorrhage

Table 1.

Comparison of Subarachnoid Hemorrhage and Control Groups

	Subarachnoid Hemorrhage	Control	P
Count	106	21	
Age (years; median, range)	55 (24-92)	56 (36-74)	0.69
Female	63 (59%)	18 (86%)	0.02
Repair Procedure			<0.0001
None	26 (25%)	0 (0%)	
Clip	16 (15%)	19 (90%)	
Coil	59 (56%)	2 (10%)	
Both Clip and Coil	1 (1%)	0 (0%)	

Platelet activation and markers of inflammation in controls and subarachnoid hemorrhage patients stratified by neurological severity

Table 2.

	All SAH	Severe EBI (HH 4-5)	Mild EBI (HH 1-3)	Controls	Overall *	p-values		
						All SAH vs Controls	Severe vs Controls	Severe vs Mild
Count [^]	106	24	82	21				
Platelet Activation (TEG MA, mm)	65.7 (52.6-79.7)	68.9 (56.9-79.7)	64.8 (52.6-76)	64.5 (56.5-60.6)	0.002	0.21	0.003	0.001
C-reactive peptide, mg/L	3.1 (0.1-255.6)	12.5 (0.5-194.4)	1.5 (0.1-255.6)	0.3 (0.1-2.0)	<0.0001	<0.0001	<0.0001	0.003
% Neutrophils	80.7 (60.2-92.4)	83.5 (70.6-92.4)	80.2 (60.2-89.5)	63.0 (43.0-79.1)	<0.0001	<0.0001	<0.0001	0.18

Markers of platelet activation and inflammation are represented as the median values over the first 72 hours from ictus in SAH and pre-aneurysm repair in control patients. Data are summarized with median and range. SAH = Subarachnoid Hemorrhage, HH=Hunt-Hess, EBI=early brain injury, TEG=thromboelastography, MA=maximum amplitude

* Comparing Severe EBI vs. Mild EBI vs. controls simultaneously

[^] CRP and % neutrophil data available in 58 SAH patients and 18 controls

Table 3.

The association with platelet activation and inflammation over the first 72 hours from aneurysm rupture with 3- month mortality and functional outcome.

	3-month mortality		3-month dichotomized modified Rankin Scale (mRS)		
	Alive	Dead	P	mRS 0-3	mRS 4-6
Count*	78	14		74	18
Platelet Activation (TEG MA, [mm])	65.3 (56.9-76.0)	68.9 (61.0-79.7)	0.01	64.9 (56.9-76.0)	68.9 (61.0-79.7)
C-reactive peptide, mg/L	2.4 (0.1-255.6)	31.1 (0.1-101.2)	0.25	1.9 (0.1-255.6)	52.4 (0.1-194.4)
%neutrophils	80.5 (69.3-91.0)	83.1 (70.6-92.4)	0.47	80.2 (69.3-89.5)	84.5 (70.6-92.4)

Data are summarized with median and range

* CRP together with 3-month mRS was available in only 50 patients and %neutrophils together with mRS was available in only 40 patients